Article

Implantation failures: success of assisted hatching with quarter-laser zona thinning

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Abstract

Implantation failure after IVF is one of the factors associated with a reduced chance of pregnancy for some patients. Assisted hatching methodologies are designed to facilitate the embryo's escape from the zona pellucida, and this strategy has been suggested as a means of improving pregnancy rates in patients with previous implantation failure. The aim of this prospective and randomized study was to evaluate the efficacy of quarter-laser zona thinning assisted hatching (qLZT-AH) in improving the implantation of embryos in patients with previous implantation failure. A total of 150 patients with a history of previous implantation failure were treated with intracytoplasmic sperm injection, and allocated into two groups: group 1, only one previous implantation failure, and group 2, repeated implantation failures. The patients in each group were randomized at the time of embryo transfer into a control group (no qLZT-AH) or experimental group where qLZT-AH was performed. For patients with repeated implantation failures, the implantation rate in those who received laser-thinned embryos was significantly higher ($P = 0.02$) than in those whose embryos were not laser-thinned (10.9 and 2.6% respectively). However, this difference was not observed in patients who presented with only one previous implantation failure. The data demonstrate that qLZT-AH is an effective strategy for improving the implantation of embryos in patients with repeated implantation failures.

Keywords: *implantation failure, quarter-laser zona thinning and assisted hatching*

Introduction

The embryo hatches from the zona pellucida (ZP) at the blastocyst stage several days after fertilization, and then begins the process of implantation. Hatching from the ZP is achieved through progressive mechanical expansion and contraction of the blastocyst, which allows the ZP to dissolve and rupture (Stein *et al.*, 1995). Successful hatching of the embryo is thought to be a key event in the process of implantation, and impaired hatching has been suggested as one of the possible factors responsible for the relatively low implantation rates of embryos resulting from IVF. The inability of embryos to hatch may be one of the major causes of embryo wastage in patients with repeated implantation failure. For more than a decade, thinning or opening of the ZP using chemical, mechanical, enzymatic or laser methods has been carried out as a method of assisting blastocyst hatching in order to favour implantation (Cohen, 1991; Gabrielsen *et al.*, 2004). Meta-analyses of randomized trials have shown a significant benefit of assisted hatching (AH) on clinical pregnancy (Edi-Osagie *et al.*, 2003) and implantation rates (Sallam *et al.*, 2003), especially in sub-groups of women with repeated previous failures of assisted conception. However, the studies are heterogeneous, and clarifying the optimum strategy for performing AH remains elusive. The aim of the present study was to evaluate, in a prospective and randomized clinical trial, the efficiency of quarter-laser zona thinning assisted hatching (qLZT-AH) as a means of improving the implantation of embryos in patients with previous implantation failure.

Materials and methods

Patient selection

According to a protocol approved by the Institutional Ethical Committee, this prospective study included a total of 150 patients who were admitted to an intracytoplasmic sperm injection (ICSI)

programme at the Human Reproduction Centre Sinhá Junqueira from January 2002 to July 2003. All of these patients presented with a history of implantation failure after transferring embryos in a previous assisted conception cycle. Patients were allocated to one of the two treatment groups, according to the number of previous implantation failures. Group 1, which included patients who presented with only one previous implantation failure after transfer of fresh embryos, was randomized into control (no qLZT-AH) and experimental (qLZT-AH performed) subgroups. Group 2 comprised patients with repeated previous implantation failures after transfer of fresh and frozen–thawed embryos (≥2 implantation failures), who were similarly randomized into control (no qLZT-AH) and experimental (qLAT-AH performed) subgroups.

Randomization into experimental and control groups was carried out first by referring to a table previously elaborated for the study, and a second randomization was then provided by drawing lots at the time of the embryo transfer procedure. Patients were allocated an identifying code number at the time of randomization in order to maintain their anonymity.

Evaluation of implantation rates was the primary outcome measure, defined as the number of gestational sacs seen on transvaginal ultrasound examination divided by the total number of embryos transferred. Clinical pregnancy, abortion, and delivery rates were calculated for each sub-group of patients (qLZT-AH and control, no qLZT-AH). A pregnancy test was performed on day 14 after embryo transfer and clinical pregnancy was determined based on ultrasound detection of a gestational sac and fetal heart beat 4 weeks after transfer.

Ovarian stimulation, oocyte retrieval, ICSI and embryo transfer cycles

All of the patients were treated with the same scheme of controlled ovarian stimulation (Franco Jr *et al.*, 2001). Down-regulation was achieved with nafarelin acetate at a dose of 400 µg/day (Synarel®; Pharmacia, São Paulo, SP Brazil), starting during the luteal phase of the previous cycle, and confirmed after 14 days of treatment. Recombinant FSH (Gonal F®; Serono, SP Brazil) was administered at a starting dose of 150–300 IU, depending on the age of the patient, for a period of 7 days. On day 8 of stimulation, follicular development was monitored by 7 MHz transvaginal ultrasound only (Medison Digital Color MT, Medison Co. Ltd, Seoul, Korea), and the FSH dose was adapted according to ovarian response. When at least three follicles measuring ≥17 mm in diameter were observed, human chorionic gonadotrophin (HCG) was administered at a dose of 5000–10,000 IU. Oocyte recovery via transvaginal ultrasound-guided aspiration was performed 36 h after HCG. Oocytes were identified from the follicular fluid, transferred into pre-equilibrated P1/3% human albumin serum (HSA) (Irvine Scientific, Santa Ana, CA, USA) and incubated at 37ºC in 5% CO_2 until denuding. Cumulus–corona removal was carried out in 40 mIU/ml hyluronidase (type IV S from bovine testes; Sigma, St Louis, MO, USA) solution in HTF/3% HSA HEPES buffered medium (Irvine Scientific). Following hyaluronidase treatment, the oocytes were assessed for maturity and all MII oocytes were subjected to ICSI, performed according to the method of Svalander *et al.* (1995). After the ICSI procedure the injected oocytes were incubated in P1/10% HSA medium. Oocytes were examined after 17–20 h to assess fertilization; those with two distinct pronuclei were considered as normal zygotes and transferred into fresh pre-equilibrated P1/10% HSA. Twenty-five to 27 h after injection, on day 1 of culture, early cleavage was evaluated and 2-cell embryos were further separated for transfer (Petersen *et al.*, 2001). Embryo quality was assessed in all patients according to the following criteria: grade 1, embryos reaching the 4-cell stage by day 2, or the 8-cell stage by day 3 with equal regular blastomeres and no fragmentation; grade 2, embryos not reaching the 4-cell stage by day 2 or 8-cell by day 3 and/or with ≤25% fragmentation; grade 3, embryos that did no reach the 4-cell stage by day 2 or the 8-cell stage by day 3 and/or ≥25% fragmentation. Embryo transfers were carried out either on day 2 or on day 3, according to the number of grade I embryos available on day 2. Day 2 embryo transfers were performed when ≤3 grade I embryos were available on day 2. For patients who had ≥3 grade I embryos on day 2, culture was extended to day 3 before transfer in order to enhance embryo selection.

Assessment of zona pellucida thickness and assisted hatching by quarter-laser zona thinning

Embryos were positioned for the assessment of ZP thickness before laser manipulation and transfer. ZP thickness measurements were performed at four points (9, 12, 3 and 6 o'clock positions), using an inverted Eclipse TE 300 microscope (Nikon Instrument, NY, USA) equipped with Hoffman lens and ocular micrometer. qLZT-AH was performed with a 1.48 µm wavelength (infrared) diode laser (Fertilase™ system; Medical Technologies Montreux, Lausanne, Switzerland) with a pilot laser light that operated through a ×40 microscope objective mounted on an inverted microscope with displacement heated stage. The embryos were treated directly in their original culture medium in 4-well tissue culture dishes (Nunc®; Nolge Products, Nunc, Denmark). The laser light was calibrated to a target spot on a video monitor as the visualization mode for aiming the laser during use. Using the displacement heated stage, the target region of the ZP in experimental embryos was positioned at this target spot on a video monitor and the laser light was fired by using a hand button to control the switch. ZP thinning was obtained by releasing a few milliseconds of laser irradiation. qLZT-AH was performed by thinning the ZP at a depth of 50–80% of the ZP thickness initiated at one point and continued until 25% of the ZP was irradiated, i.e. laser drilling was initiated at the 9 o'clock position and consecutive irradiations were generated until the 12 o'clock position was reached. Control of the aperture size depended on the irradiation time, so that a maximum of eight ablations were made successively around the zona with an irradiation time of 9 ms to reach a total length of approximately 80 µm. After the laser procedure, all embryos were transferred to fresh culture medium in order to avoid possible toxicity of products derived from the action of the laser on the organic components of the ZP. qLZT-AH was performed on the embryos of experimental groups on day 2 or day 3 (**Figure 1**).

Data analysis

Data are reported as means ± SD and were analysed using the InStat 3.0 program for MacIntosh (GraphPad Software, San Diego, CA, USA). The Mann–Whitney test and Fisher's exact test were used when appropriate. The level of significance was set at $P < 0.05$.

Results

Group 1

Table 1 summarizes the characteristics and results of patients with only one implantation failure. The mean age, the aetiology, the mean number of oocytes retrieved, number of MII oocytes, fertilization rate, embryo ZP thickness and number of embryos transferred did not differ between the control and experimental groups. Embryo quality assessment is also shown in **Table 1**. There was no significant difference in the number of grade I, II and III embryos between the zona laser-thinned and control groups. The distribution of transfers on day 2 and day 3 was similar in laser-thinned and control groups. No significant difference in implantation and pregnancy rates was observed between groups with or without AH.

Group 2

Table 2 illustrates characteristics and clinical results in patients with repeated implantation failures: no significant difference was observed in the mean of the number of previous implantation

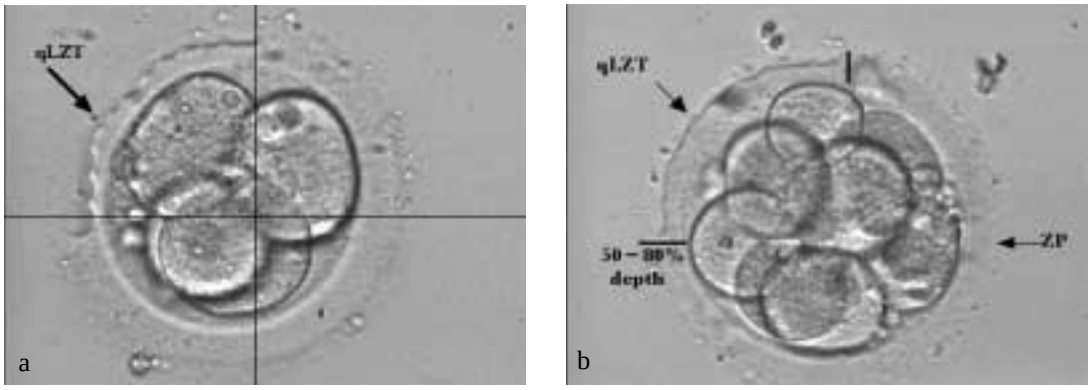

Figure 1. Zona pellucida breaching of embryos undergoing qLZT-AH under inverted microscopy at magnification of ×400. (**a**) 25% of the ZP of a 4-cell fresh embryo (day 2) is thinned at a depth of 50–80% (arrowhead). (**b**) qLZT-AH of the ZP in an 8-cell fresh embryo (day 3).

Table 1. Group 1: characteristics and clinical results of patients with one previous implantation failure. Values in parentheses represent percentages.

	qLZT-AH (experimental)	*No qLZT-AH (control)*
Patients (*n*)	35	35
Cycles (*n*)	35	35
Age (years)[a]	34.6 ± 4.6	34.1 ± 5.3
Aetiology		
Male	17 (48.6)	12 (34.3)
Female	5 (14.3)	13 (37.1)
Mixed	10 (28.5)	8 (22.9)
Idiopathic	3 (8.6)	2 (5.7)
Retrieved oocytes[a]	9.2 ± 4.5	8.1 ± 4.4
MII oocyte[a]	7.6 ± 3.9	6.5 ± 3.3
Fertilization (%)[a]	71.2 ± 21.6	73.2 ± 19.7
Quality of embryos transferred		
Grade I	55 (58)	47 (50)
Grade II	38 (40)	41 (44)
Grade III	2 (2)	6 (6)
Embryo ZP thickness (µm)[a]	16.0 ± 0.7	16.2 ± 0.5
Embryo transfer[a]	2.7 ± 0.9	2.7 ± 0.7
Pregnancies (*n*)	11	10
Implantation rate (%)	15.8	14.9
Pregnancy rate/transfer (%)	31.4	28.6
Abortions (*n*)	3	0
Deliveries (*n*)	8	10

There were no significant differences between the experimental and control groups.
[a]Values are mean ± SD.

Table 2. Group 2: general characteristics and clinical results of the patients with repeated implantation failures. Values in parentheses represent percentages.

	qLZT-AH (experimental)	*No qLZT-AH (control)*
Patients (*n*)	40	40
Cycles (*n*)	40	40
Age (years)[b]	35.7 ± 3.8	35.3 ± 5.1
Aetiology		
Male	15 (37.5)	17 (42.5)
Female	11 (27.5)	18 (45)
Mixed	10 (25)	3 (7.5)
Idiopathic	4 (10)	2 (5)
Implantation failures		
2	16 (40)	22 (55)
3	12 (30)	12 (30)
4	5 (12.5)	3 (7.5)
5	5 (12.5)	2 (5)
6	2 (5)	0
7	0	1(2.5)
Retrieved oocytes[b]	9.7 ± 6.1	9.5 ± 5.7
MII oocyte[b]	8.1 ± 5.2	7.6 ± 3.9
Fertilization (%)[b]	74.4 ± 20.8	70.9 ± 17.0
Quality of embryos transferred		
Grade I	48 (40.3)	53 (46.5)
Grade II	56 (47.1)	54(47.4)
Grade III	15 (12.6)	7 (6.1)
Embryo ZP thickness (µm)[b]	17.6 ± 2.4	17.2 ± 2.8
Embryo transfer[b]	3.0 ± 0.9	2.9 ± 0.8
Pregnancies (*n*)	10	03
Implantation rate (%)	10.9[a]	2.6[a]
Pregnancy rate/transfer (%)	25.0	7.5
Abortion (*n*)	1	0
Deliveries (*n*)	8	3

[a]Values were significantly different (P = 0.02).
[b]Values are mean ± SD.

failures between qLZT-AH and control groups (3.2 ± 1.2 and 2.8 ± 1.1 respectively). The mean age, the mean number of oocytes retrieved, MII oocytes, fertilization rate, embryo ZP thickness and number of embryos transferred was not significantly different between the AH and control groups. There was no significant difference in the number of grade I, II and III embryos between laser-thinned or control groups. The distribution of transfers on day 2 and day 3 was similar in both groups. In contrast to patients with only one implantation failure, the implantation rate of laser-thinned embryos was significantly higher ($P = 0.02$) than the control group (10.9 and 2.6% respectively). Pregnancy rate was not significantly different between laser-thinned and control groups.

Discussion

Patients with unexplained previous IVF failure have a reduced chance of pregnancy in subsequent treatment cycles. The mechanisms that have been suggested to explain their repeated failure include zona hardening, asynchrony between the embryo and the endometrial implantation window after ovarian stimulation, and a deficiency in the cellular energy required for hatching (Schoolcraft *et al.*, 1994). Retrospective trials suggest either that AH is of benefit for patients with repeated previous implantation failure (Obruca *et al.*, 1994; Takahashi *et al.*, 1994; Stein *et al.*, 1995; Parikh *et al.*, 1996; Magli *et al.*, 1998) or have shown no benefit (Edirisinghe *et al.*, 1999). The few randomized published studies have been performed with a limited number of patients, using a variety of methods, making interpretation of the findings difficult (Antinori *et al.*, 1996; Chao *et al.*, 1997; Rufas-Sapir *et al.*, 2004). However, meta-analysis of some of these randomized studies has demonstrated an improvement in pregnancy rates with AH, especially for women with repeated previous implantation failures, suggesting that AH probably does enhance pregnancy in these patients (Edi-Osagie *et al.*, 2003). In addition, a recent European multicentre prospective study (Primi *et al.*, 2004) has suggested that failure of implantation after several transfers of good quality embryos remains the strongest patient selection criterion for AH.

The methodology used for AH in relation to assisted reproduction outcomes continues to be discussed. There is no consensus regarding the possible advantage of breaching or thinning the ZP in order to achieve a higher frequency of blastocyst hatching and higher implantation rate. Making a hole in the ZP aids blastocyst hatching. However, this procedure may also have detrimental effects, such as allowing escape of blastomeres, premature hatching without blastocyst expansion, or potential risks introduced because of a lack of protection by the ZP. Thinning the ZP has the advantage of retaining the zona to protect the embryo, with a reduced risk of damaging the blastomeres during manipulation. Laser methodology has progressively replaced the previous AH methodologies, as it allows rapid, controlled and safe microdissection of the ZP (Rink *et al.*, 1996; Malter *et al.*, 2001; Benjamin *et al.*, 2003). This technique has also been used for micromanipulation of human oocytes, AH and polar body and embryo biopsy (Veiga *et al.*, 1997, Baruffi *et al.* 2000; Nagy *et al.*, 2002; Petersen *et al.*, 2002; Montag *et al.*, 2004).

Thinning the human ZP over an area as large as one quarter with the use of a laser has been shown to be effective in 'in-vitro hatching' (Blake *et al.*, 2001). It has also been shown that transfer of embryos with high variation in ZP thickness may result in higher pregnancy rates (Gabrielsen *et al.*, 2000, 2001). A low variation in ZP thickness has been shown to correlate with advanced female age, elevated FSH, and suboptimal embryo quality (Host *et al.*, 2002). Mantoudis *et al.* (2001) has shown better implantation and pregnancy rates with qLZT-AH methodology compared with the total breaching of the ZP for a patient population that presents with at least one of the following criteria: advanced age, two previous implantation failures, a requirement for high gonadotrophin doses, and patients having frozen embryo replacement; no control group was included in their study. In the present study, the implantation rate after using the qLZT-AH methodology was significantly higher compared with control (10.9 versus 2.6%, $P = 0.02$ respectively), supporting the idea that this specific methodology is efficient for patients with repeated previous implantation failures. However, the results did not show an improvement in implantation with the use of qLZT-AH for patients with only one previous implantation failure (15.8 versus 14.9% respectively). This observation allows us to suggest that patients who fail to implant only once may have other factors, such as features of the embryo or endometrium, that affect implantation potential. However, there are no studies describing the efficiency of AH in patients with only one implantation failure, and further studies with large numbers of patients are needed to confirm the efficacy of AH in this specific population. In addition, the quality of embryos transferred is an important bias in evaluating the results of implantation. In the present study, there was no significant difference in the quality distribution of embryos transferred (grade I, II and II) in qLZT-AH and control groups of subgroups I and II. In addition, the clinical pregnancy rate was not significantly different in either group (group I: qLZT-AH = 31.4% versus control = 28.6%, group II: qLZT-AH = 25% versus control = 7.5%). Accepting a null (Ho) hypothesis (no difference between groups) when the hypothesis in reality is false would induce a so-called type II error (β-error), and it is advisable to increase the number of patients studied in order to avoid this type of error. A total of 8554 patients (group I) and 160 patients (group II) would be required in order to detect a difference in the pregnancy rates between qLZT-AH and control groups, with a power of 80% and significance level of 5%.

In conclusion, the data indicate that qLZT-AH is of benefit in patients with repeated implantation failures (≥2 previous implantation failures). As far as is known, this is the first report that includes control groups, and a larger study group is necessary to confirm this observation.

References

Antinori S, Selman HA, Caffa B *et al.* 1996 Zona opening of human embryos using a non-contact UV laser for assisted hatching in patients with poor prognosis of pregnancy. *Human Reproduction* **11**, 2488–2492.

Baruffi RL, Mauri AL, Petersen CG *et al.* 2000 Zona thinning with noncontact diode laser in patients aged < or = 37 years with no previous failure of implantation: a prospective and randomized study. *Journal of Assisted Reproduction and Genetics* **17**, 557–560.

Benjamin CW, Boyd CA, Lanzendorf SE 2003 Randomized controlled study of human zona pellucida dissection using the zona infrared laser optical system: evaluation of blastomere damage, embryo development, and subsequent hatching. *Fertility Sterility* **80**, 1249–1254.

Blake DA, Forsberg AS, Johansson BR, Wikland M 2001 Laser zona pellucida thinning – an alternative approach to assisted hatching. *Human Reproduction* **16**, 1959–1964.

Chao KH, Chen SU, Chen HF *et al.* 1997 Assisted hatching increases the implantation and pregnancy rate of in vitro fertilization–embryo transfer, but not that of IVF tubal embryo transfer in patients with repeated IVF failures. *Fertility and Sterility* **67**, 904–908.

Cohen J 1991 Assisted hatching of human embryos. *Journal of In Vitro Fertility and Embryo Transfer* **8**, 179–190.

Edi-Osagie E, Hooper L, Seif MW 2003 The impact of assisted hatching on live birth rates and outcomes of assisted conception: a systematic review. *Human Reproduction* **18**, 1828–1835.

Edirisinghe WR, Ahnonkitpanit V, Promviengchai S *et al.* 1999 A study failing to determine significant benefits from assisted hatching: patients selected for advanced age, zona thickness of embryos, and previous failed attempts. *Journal of Assisted Reproduction and Genetics* **16**, 294–301.

Franco Jr JG, Baruffi RLR, Coelho J *et al.* 2001 Prospective randomized comparison of ovarian blockade with nafarelin versus leuprolide during ovarian stimulation with recombinant FSH in an ICSI program. *Journal of Assisted Reproduction and Genetics* **18**, 593–597.

Gabrielsen A, Bhatnager PR, Petersen K, Lindenberg S 2000 Influence of zona pellucida thickness of human embryo on clinical pregnancy outcome following in vitro fertilization treatment. *Journal of Assisted Reproduction and Genetics* **17**, 323–328.

Gabrielsen A, Lindenberg S, Petersen K 2001 The impact of the zona pellucida thickness variation on human embryos on pregnancy outcome in relation to suboptimal embryo development. A prospective randomized controlled study. *Human Reproduction* **16**, 2166–2170.

Gabrielsen A, Agerholm I, Toft B *et al.* 2004 Assisted hatching improves implantation rates on cryopreserved–thawed embryos. A randomized prospective study. *Human Reproduction* **19**, 2258–2262.

Host E, Gabrielsen A, Lindenberg S, Smidt-Jensen S 2002 Apoptosis in human cumulus cells in relation to zona pellucida thickness variation, maturation stage, and cleavage of corresponding oocyte after intracytoplasmic sperm injection. *Fertility and Sterility* **77**, 511–515.

Magli MC, Gianaroli L, Ferraretti AP *et al.* 1998 Rescue of implantation potential in embryos with poor prognosis by assisted zona hatching. *Human Reproduction* **13**, 1331–1335.

Malter HE, Schimmel T, Cohen J 2001 Zona dissection by infrared laser: developmental consequences in the mouse technical considerations, and controlled clinical trial *Reproductive BioMedicine Online* **3**, 117–123.

Mantoudis E, Podsiadly BT, Gorgy A *et al.* 2001 A comparison between quarter, partial and total laser assisted hatching in selected infertility patients. *Human Reproduction* **16**, 2182–2186.

Montag M, Van Der Ven K, Dorn C, Van der Ven H 2004 Outcome of laser-assisted polar body biopsy aneuploidy testing. *Reproductive BioMedicine Online* **9**, 425–429.

Nagy ZP, Oliveira SA, Abdelmassih V, Abdelmassih R 2002 Novel use of laser to assisted ICSI for patients with fragile oocytes: a case report. *Reproductive BioMedicine Online* **4**, 27–31.

Obruca A, Strohmer H, Sakkas D *et al.* 1994 Use of lasers in assisted fertilization and hatching. *Human Reproduction* **9**, 1723–1726.

Parikh FR, Kamat SA, Nadkarni S *et al.* 1996 Assisted hatching in an in vitro fertilization programme. *Journal of Reproduction and Fertilility Supplement* **50**, 121–125.

Petersen CG, Mauri AL, Baruffi RL *et al.* 2002 Zona thinning with noncontact diodo laser in ICSI embryos from women of advanced age. *Journal of Assisted Reproduction and Genetics* **19**, 512–516.

Petersen CG, Mauri AL, Ferreira R *et al.* 2001 Embryo selection by the first cleavage parameter between 25 and 27 h after ICSI. *Journal of Assisted Reproduction and Genetics* **18**, 209–212.

Primi MP, Senn A, Montag M *et al.* 2004 A European multicenter prospective randomized study to assess the use of assisted hatching with diode laser and the benefit of an immunosuppressive/antibiotic treatment in different patient populations. *Human Reproduction* **19**, 2325–2333.

Rink K, Delacretaz G, Salathe RP *et al.* 1996 Non-contact microdrilling of mouse zona pellucida with an objective-delivered 1.48-microns diode laser. *Lasers Surgery Medical* **18**, 52–62.

Rufas-Sapir O, Stein A, Orvieto R *et al.* 2004 Is assisted hatching beneficial in patients with recurrent implantation failures? *Clinical Experimental Obstetrics and Gynecology* **31**, 110–112.

Sallam HN, Sadek SS, Agameya AF 2003 Assisted hatching – a meta analysis of randomized controlled trials. *Journal of Assisted Reproduction and Genetics* **20**, 332–342.

Schoolcraft WB, Schlenker T, Gee M, Jones Jr HW 1994 Assisted hatching in the treatment of poor prognosis in vitro fertilization candidates. *Fertility and Sterility* **62**, 551–554.

Stein A, Rufas O, Amit S *et al.* 1995 Assisted hatching by partial zona dissection of human pre-embryos in patients with recurrent implantation failure after in vitro fertilization. *Fertility and Sterility* **63**, 838–841.

Svalander P, Forsberg AS, Jakobsson AH, Wikland M 1995 Factors of importance for the establishment of a successful program of intracytoplasmic sperm injection treatment for male infertility. *Fertility and Sterility* **63**, 828–837.

Takahashi K, Takenaka M, Ishizuka B 1994 The effect of assisted hatching on patients repeatedly failed to conceive by in vitro fertilization. *Nip S F G Zasshi* **16**, 1009–10012.

Veiga A, Sandalinas M, Benkhalifa M *et al.* 1997 Laser blastocyst biopsy for preimplantation diagnosis in the human. *Zygote* **5**, 351–354.

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